Award Number:
W81XWH-14-1-0360

TITLE:
Targeting ESR1-Mutant Breast Cancer

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REPORT DATE:
September 2015

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
September 2015

2. REPORT TYPE
Annual

3. DATES COVERED
1 Sep 2014 - 31 Aug 2015

4. TITLE AND SUBTITLE
Targeting ESR1-Mutant Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0360

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The hypothesis of this proposal is that LBD mutations in ESR1 promote resistance to current FDA approved hormonal therapies and that more potent, selective estrogen receptor degraders (SERDs) will enable complete inhibition of mutant ER signaling and thus have substantial therapeutic benefit. Our first aim in this proposal was to determine the biochemical and biologic impact of ER mutations found in breast cancer using both structural and cell based assays. We have now have evidence for the effects of the most recurrent mutations, D538G and Y537S inducing an agonist apo-ER structure and promoting estrogen independent tumor growth as well as preliminary evidence for the mutants promoting transcriptional effects including but also beyond those induced by estrogen. Additional aims were to understand how mutant ER is impacted by SERD compounds again using structural, cell-based, and mouse models as readouts. We have evidence that the Y537S and D538G alterations modify the SERM (4OHT) induced antagonist structure to a greater extent than SERDs (bazedoxifene), suggesting SERDs as potentially superior agents. Our biologic data from a suite of ER antagonists suggest the same, that SERDs such as bazedoxifene or GDC0180 potently inhibit wild type and mutant ER driven phenotypes. Overall, the work is suggesting rational steps forward towards targeting ER mutant driven cancers

15. SUBJECT TERMS

Nothing listed

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	13	**19b. TELEPHONE NUMBER** ***(include area code)***

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. Introduction

The estrogen receptor alpha (ERα) is critical for the etiology and treatment for the majority of breast cancers. While antiestrogen therapies have dramatically increased disease-free survival, the majority of breast cancer patients will present recurrent antiestrogen resistant metastatic lesions following prolonged exposure to these therapies. By investigating how these lesions become resistant to antiestrogen while maintaining expression of ERα, we found mutations to the ERα ligand binding domain (LBD) in a significant population of patients who previously received antiestrogen therapies. Importantly, these mutations confer hormone-free transcriptional activity and reduced antiestrogen potency onto ERα. The overriding hypothesis of this proposal is that novel selective estrogen receptor degraders (SERDs) with improved potencies will have significant utility at targeting breast cancers presenting these mutations and will offer substantial increases in disease-free survival to the patient. This proposal integrates murine xenograft, breast cancer cell line, biochemical and structural biology techniques to uncover the best candidate drugs for the clinical targeting of these mutants.

2. Keywords

Estrogen Receptor
Acquired Drug Resistance
Metastatic Breast Cancer
Selective Estrogen Receptor Modulator (SERM)
Selective Estrogen Receptor Degrader (SERD)
Tamoxifen
Fulvestrant
Raloxifene
Bazedoxifene
Ligand Binding Domain
Structure

3. Accomplishments

The **Year 1** goal of this project was to determine how S463P, Y537S and D538G ERα mutations alter the structure of the ligand binding domain (LBD) such that it escapes hormone regulation and resists antagonism by SERM and SERD. Towards that end, we obtained x-ray crystal structures for the D538G mutant in the apo (unliganded), estradiol (hormone)-bound, and 4-hydroxytamoxifen (4-OHT, SERM)-bound states. X-ray crystal structures for the Y537S mutant in the apo and hormone-bound states were previously published. These structures show that the Y537S mutant adopts the activated (agonist) state in the absence of hormone by forming a hydrogen bond between S537 and D351. This interaction remains intact when hormone is bound. Therefore these structures show that the Y537S adopts a stable agonist conformation in the absence of hormone that enables it to achieve a high level of transcriptional activity within the breast cancer cell. For the D538G, our structures show that the mutant adopts the agonist conformation in the absence of hormone through a more subtle mechanism that accounts for its reduced constitutive activity in breast cancer cells relative to Y537S. Specifically, the D538G mutation reduces the conformational strain on the H11-12 loop that is observed in the WT-estradiol (E2) crystal structure. Interestingly, this region appears flexible in the apo D538G because it adopts two different conformations between the two monomers in the asymmetric unit. Further, addition of E2 stabilizes the loop in the same conformation in each monomer in the asymmetric unit (**Figure 1**). Together these data agree with the reported activities of the mutant receptors whereby the apo D538G appears less stable and has reduced transcriptional activity compared to Y537S. When E2 is added to the D538G mutant, it stabilizes the receptor and the transcriptional activity matches that of the WT with E2.

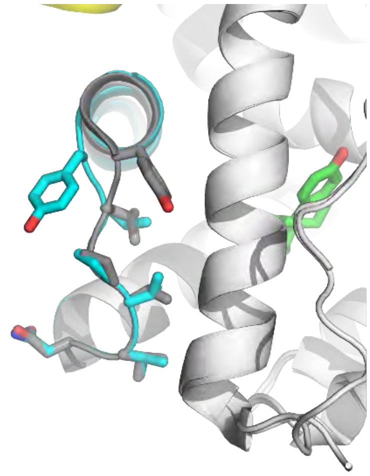

Figure 1: Superposition of WT-E2 and (Grey) and D538G-E2 (Cyan) ERα LBD complex crystal structures. Residues comprising the H11-12 loop are shown as side-chain sticks. Estradiol is shown as green sticks.

Next, we used x-ray crystal structure analysis and atomistic molecular dynamics simulations to reveal structural changes to the antagonist conformation in the Y537S and D538G mutations. WT structures were previously solved for 4-hydroxytamoxifen (4-OHT) and raloxifene (RAL). Bazedoxifene is a newer mixed SERM/SERD that has potential utility at inhibiting the somatic mutations. As such, we solved an x-ray crystal structure for the wild-type (WT) ERα in complex bazedoxifene. For the mutant structures, we obtained an x-ray crystal structure of 4-OHT bound to the D538G mutant. Atomistic molecular dynamics simulations (MD SIM) were used to predict the structures of Y537S in complex with RAL, 4-OHT and BZA. MD SIM was also used to predict the structures of D538G with RAL and BZA. We found that, for the WT, BZA possesses SERD-properties because it interferes with the helix 11-12 loop to disorder helix 12 relative to RAL. For the mutants, we found that for each of the SERMs examined, there appears to be conformational changes at the helix 11-12 loop and helix 12 (**Figure 2**). We believe that these changes show that the molecules bind

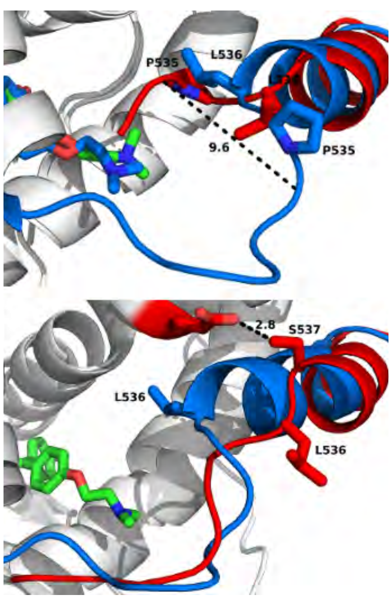

Figure 2: Top) Superposition of WT (Blue) and D538G (Red) in complex with 4-OHT ERα LBD. Bottom) Superposition of WT (Blue) with Y537S in complex with 4-OHT

with a reduced affinity and likely make the receptors resistant to proteolytic degradation, an important aspect of SERM/SERD potency.

Finally, we obtained x-ray crystal structures of the 463P mutant in complex with E2 and 4-OHT in order to determine how the mutation impacted ERα LBD structure. Unfortunately, the 463P region was artificially influenced by crystal contacts in the E2 bound structure. Fortunately, the 4-OHT complex structure crystallized in a different space group where the region was not artificially influenced. Interestingly, while the 463 loop is usually disordered in ERα LBD structures, here it is ordered and appears to clamp over the dimer interface (**Figure 3**). As such, this structure shows that the 463P appears to stabilize the ERα LBD homodimer interface.

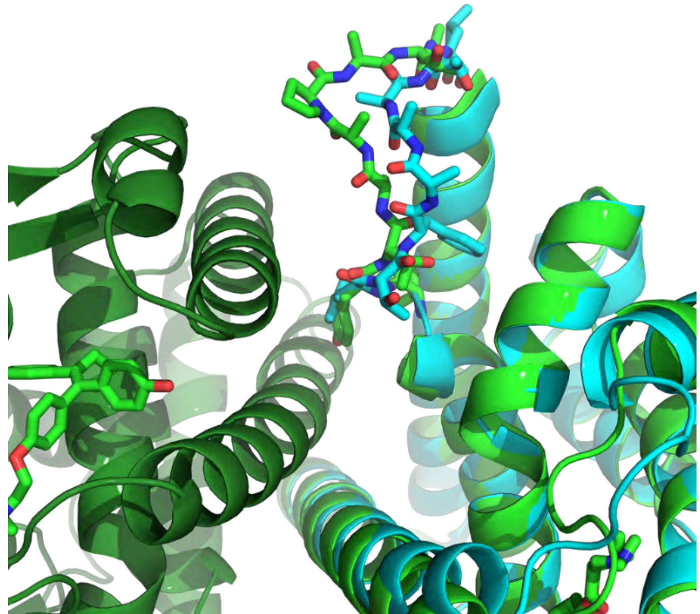

Figure 3: Superposition between the 463P-4-OHT complex and WT-4-OHT (PDB: 3ERT). 463P is shown in green and WT in cyan. The dimer partner of 463P is dark green.

B) Opportunities for training and professional development

Training and professional development were not stated goals of this project.

C) Dissemination of results to community

Dr. Fanning has reported results at the San Antonio Breast Cancer Conference and the University of Chicago Biomedical Sciences Cluster Retreat, and he has been invited to speak at the upcoming 3rd Congress on Steroid Research.

The following manuscripts are in preparations for publication:

1) "*ESR1* Somatic Mutations Y537S and D538G Confer Breast Cancer Endocrine Resistance by Stabilizing the Active AF-2 conformation of ERα" has been submitted to *eLife*.
2) "Bazedoxifene Potently Inhibits Helix 12 ESR1 Somatic Mutations by Disrupting the Constitutively Active AF-2 Conformation" is in preparation and will be submitted October 2015.

D) Plans for upcoming reporting period

The project plans for Year #2 are in line with our initial Statement of Work. We are screening crystallographic conditions for next generation SERM/SERDs, such as AZ9496, in complex with the WT and mutant ERα LBDs to determine how they interact with the proteins. Additionally, we are screening crystallographic conditions to reveal the structure of the E380Q mutant in the apo, hormone-bound, and SERM/SERD-bound states. Further we are pursuing biophysical and biochemical assays to uncover differences in hormone/drug binding affinities, coregulator binding affinities, dimerization, and overall stability of the protein.

4. Impact

A) Impact on the development of the principal discipline

To date, the major impact of this work has been significant biological, biochemical and structural characterization of somatic mutations in ERα that arise in ~30% of hormone/antiestrogen resistant metastatic breast cancers. We have shown that the two most prevalent mutations, Y537S and D538G, escape hormone regulation by conferring transcriptional activity onto ERα in the absence of estrogen. This dysfunctional intrinsic activity arises from the ability of the mutants to adopt the agonist conformation in the absence of hormone. This explains why aromatase inhibitors, which act by reducing endogenous estrogen levels, are ineffective against these mutants. Importantly, the release of these structures through the PDB, thereby making them available to the public, will enable researchers from around the world to develop novel SERDs that target the Y537S and D538G mutants. The impact of this work will arise from the novel compounds developed and brought to the clinic for testing to replace the ones that are rendered ineffective by these somatic mutations.

B) Impact on other disciplines

Nothing to report

C) Impact on technology transfer

Nothing to report

D) Impact on society beyond science

Nothing to report

5. Changes

Our considerable success in obtaining ER LBD x-ray crystal structures required re-allocating funds originally earmarked for computational costs to protein expression, biochemical and structural studies. Extensive modeling and simulation studies were, however, carried out by our major collaborator Yang Shen, who has since moved to Texas A&M University and used other funds for computational studies, and by Chris Mayne, who is a member of the Beckman Institute for Advanced Science and Technology at the University of Illinois and also used other funding. Going forward, we will continue to use reallocated DOD funds for structural, biophysical and biochemical studies of mutant ERs, which we have expanded to take advantage of our success thus far. Drs. Shen and Mayne will continue to assist us from their respective institutions without the need for funds allocated to this project.

6. Products

A) Journal Publications

We are about to submit a manuscript to *eLife* and are preparing another for submission within the next few months. Site#1 has a separate manuscript in preparation.

B) Presentations

1) GG: Northwestern Universtiy, Chicago IL – invited seminar speaker

2) GG: Breast Cancer Think Tank Meeting, Grand Cayman, BWI – symposium speaker

3) GG: Delhi Center workshop, Delhi India – invited speaker

4) GG: Pfizer TSEC Advisory Board meeting, Clearwater, FL – symposium speaker

5) GG: PacRim meeting – Stevenson, WA – symposium speaker

1) SWF: San Antonio Breast Cancer Symposium . Poster presentation.

2) SWF: University of Chicago Biomedical Sciences Cluster Retreat. Invited oral presentation.

C) Other products

Nothing to report.

7. Participants

Name: Geoffrey L. Greene

Project role: PI

Researcher identifier:

Nearest person work month:

Contribution to project: Dr. Greene supervised the laboratory work described in this project including experiment design, interpretation, and reporting.

Other Funding Support:

Virginia and D.K. Ludwig Fund for Cancer Research, NIH P30, NCI 5T32, Prostate Cancer Foundation

Name: Sean W. Fanning

Project role: Postdoctoral Researcher

Researcher identifier:

Nearest person work month:

Contribution to project: Dr. Fanning conducted the laboratory work described in this project including experimental design, execution, and interpretation and reporting.

Other Funding Support: Susan G. Komen Foundation

Name: Bradley Green

Project Role: Research Technologist

Researcher identifier:

Nearest person work month:

Contribution to the project: Mr. Green assisted Dr. Fanning in the execution of experiments.

Other Funding Support:

Virginia and D.K. Ludwig Fund for Cancer Research

Other Collaborating Organizations

Name: Yang Shen

Project Role: Collaborator

Institution: Texas A&M University

Contribution to the project: Dr. Shen provided molecular dynamics simulations and assisted in reporting the findings of our work.

Name: Christopher G. Mayne

Project Role: Collaborator

Institution: Beckman Institute for Advanced Science and Technology, the University of Illinois Urbana-Champaign

Contribution to the project: Dr. Mayne provided molecular dynamics simulations and assisted in reporting the work.

8. Special Reporting Requirements

Please refer to the separate report from Site#1 PI – Dr. Sarat Chandarlapaty

9. Appendices

Nothing to report